
Conformational Analysis of Nucleic Acid Molecules with Flexible Furanose Rings in Dihedral Angle Space

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ABSTRACT

The computational algorithm that works in the coordinate space of dihedral angles (i.e., bond lengths and bond angles are kept fixed and only rotatable dihedral angles are treated as independent variables) is extended to deal with the pseudorotational motion of furanose rings by introducing a variable of pseudorotation. Then, this algorithm is applied to a distance geometry calculation that generates three-dimensional (3D) structures that are consistent with given constraints of interatomic distances. This method efficiently generates 3D structures of an RNA hairpin loop which satisfy a set of experimental NMR data. © 1996 by John Wiley & Sons, Inc.

Introduction

Computational chemistry has made various contributions to conformational analyses of macromolecules. Many computer programs have been developed to analyze their static and/or dynamic conformations at the atomic level. They can be classified into two types from the viewpoint of independent variables used to describe conformations of macromolecules: one in which Cartesian

coordinates are independent variables, for example, CHARMM,¹ AMBER^{2,3} and DSPACE,⁴ and the other in which bond lengths and bond angles are kept fixed and only rotatable dihedral angles are treated as independent variables, for example, ECEPP,^{5,6} FEDER,⁷ and DADAS.⁸ Although the computer programs of the latter type take more complicated structures, they have the advantage of being more computationally efficient because of a smaller number of independent variables.⁷ It proved actually to be very powerful as applied to proteins for which the number of independent variables in the dihedral angle space (DAS) is

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about one-eighth of the corresponding number in Cartesian coordinate space (CCS).⁸⁻¹⁰

Therefore, it is desirable to extend the programs so that nucleic acids can also be treated. The programs are developed on basic assumption that a molecule has a topology of tree, i.e., does not have any flexible rings in it.⁷ Accordingly, they cannot be applied directly to nucleic acids, unless furanose rings are treated as rigid. In fact, such a treatment has actually been done recently for distance geometry calculation¹¹ and normal mode analysis of nucleic acids.¹² Furanose rings have important roles of making up the backbone of nucleic acid molecules together with phosphate groups and of providing attachment points for bases. Since planar conformations of five-membered rings are considerably strained, they take various puckered conformations. Changes among them called pseudorotation are known to have significant influence on overall molecular conformations of nucleic acids and on their dynamics. Therefore, truly successful programs for nucleic acids in DAS should allow pseudorotational motion of furanose rings. However, this can be possible only by overcoming the problem of the basic assumption of the tree topology. Proteins also have a similar problem in proline rings. For this problem, Abagyan et al. proposed one solution which involves linear algebraic equations among variables of rings for ring closure.¹³ In the present study, we solve the problem by introducing the variable of pseudorotation and develop an efficient algorithm for nucleic acids.¹⁴ This leads to considerable reduction of the number of independent variables as compared with that in CCS; the ratio of them results in about one-eleventh for nucleic acids.

Then, we apply this algorithm to a distance geometry calculation which generates three-dimensional (3D) molecular conformations that are consistent with a given set of constraints of interatomic distances deduced by NMR experimental data. Several distance geometry algorithms have been proposed and are widely used. They also can be classified into two basic types: one based on the metric matrix method¹⁵⁻¹⁷ and the other based on the variable target function method.⁸ Although the metric matrix method that works in CCS has been used widely for proteins^{18,19} and applied to nucleic acids,²⁰⁻²² its efficient use is limited to small or simplified structures, because, for larger molecules, it requires a large amount of computing time. The variable target function method, which generates satisfactory conformations by minimiz-

ing a target function in DAS, is more powerful in this respect.⁸ However, its usefulness as applied to nucleic acids has been limited because of the difficulty of treating flexible furanose rings.

In the present paper, we apply the algorithm to the determination of 3D conformations of an RNA hairpin loop whose sequence is 5'GGAC(UUCG)GUCC from a set of experimental NMR data, and examine its power and validity as a distance geometry algorithm for nucleic acids.

Method

VARIABLES

Any macromolecule should be regarded as having a tree structure consisting of rigid units and rotatable bonds connecting the units in order to treat only dihedral angles as independent variables. The tree structure means that a molecule has no loop with rotatable covalent bonds.⁷ Because of this treatment, furanose rings in nucleic acids had hitherto to be treated as rigid units in DAS. However, if we can conceptually treat one of the bonds in a ring as being cut, then we can treat nucleic acid molecules as having a tree structure. This can be done if we can cut the bond, but still keep the distance between the two atoms of the cut bond always and exactly to its standard value. This requirement can be satisfied by also allowing bond angles in the ring to change and to impose some functional relationship among bond angles and dihedral angles in the ring.

Conformational change among their various puckered states in five-membered saturated rings, e.g., cyclopentane, tetrahydrofuran, ribose, and deoxyribose, is called pseudorotation and has been studied well.^{14,23-27} Recently two of the authors of this paper developed an analytic theory in which five bond lengths are kept fixed, and values of five bond angles and five dihedral angles in various puckered conformations are given as explicit functions of the pseudorotation variable ω ¹⁴:

$$\theta_j^{\text{dep}} = \theta_j(\omega) \quad (1)$$

An example of such explicit functions is given for case of ribose rings. Five bond angles θ_j ($j = 0, 1, \dots, 4$) and five dihedral angles φ_j ($j = 0, 1, \dots, 4$) are given by

$$\cos \theta_j = a_j + b_j \cos[2\{\omega + 4/5(j-1)\pi + c_j\pi\}] \quad (j = 0, 1, \dots, 4) \quad (2)$$

$$\cos \varphi_i = \frac{1 - 2 \cos \theta_i + 2 \cos \theta_{i+2} - 2 \cos \theta_{i+4} + 2 \cos \theta_i \cos \theta_{i+4}}{2 \sin \theta_i \sin \theta_{i+4}} \quad (i = 0, 1, \dots, 4) \quad (3)$$

where suffix i in variable θ_i is to be understood to mean $i - 5$ when $i > 5$. Values of parameters a_j , b_j , and c_j , which have been determined by assuming the AMBER potential² for intraring interactions, are listed in Table I.

In these expressions, a series of conformations of a furanose ring is generated by a change of the pseudorotation variable ω from 0 to 2π . Consequently, we can treat this pseudorotation variable as an independent variable. Introduction of this new type of variables leads us to define a new concept of variable units in the tree topological molecular structure (Figure 1). Changes of ordinary independent variables lead to rotations about rotatable bonds, whereas in the variable units, change of a pseudorotation variable brings about simultaneous variations of all dependent variables according to eq. (1). Even though we have given an example of eqs. (2) and (3) in the above, we will develop a formulation in the following based on a general expression of eq. (1). Therefore, the method developed here is applicable for molecules containing any five-membered rings.

In order to treat nucleic acid molecules to have a tree topological structure, we introduce a conceptual cut in a furanose ring, e.g., between bond C_4-O_4 (Figure 2). However, we let all bond angles and dihedral angles in the ring have values given by eq. (1), so that the length of the cut bond C_4-O_4 is indeed maintained in its standard value.

With the above trick of introducing a conceptual cut and requiring eq. (1) for new variables, we can treat nucleic acid molecules as having a tree topology. However, we now have bond angles as

our new variables. Because our basic formulation has been developed in DAS, we again introduce a trick to treat bond angles as if they were dihedral angles. We do so by introducing pseudoatoms that have no mass, no volume, and no interaction (Figure 2). Here we will explain the trick by taking bond angle θ_3 as an instance. Two pseudoatoms are introduced into the bond between C_2 and C_3 atoms: One marked P_1 in Figure 2 is placed on an axis perpendicular both to C_2-C_3 and C_3-C_4 bonds, and another marked P_2 is placed on the middle position between C_2 and C_3 atoms. By viewing C_4 , C_3 , P_1 , P_2 , and C_3 as main-chain atoms, the bond angle θ_3 is now nothing but a dihedral angle about the "backbone bond" of C_3-P_1 . When bond C_4-O_4 is cut, the six "dihedral angles" in Figure 2 have to be treated as new variables.

With these considerations, nucleic acid molecules with flexible furanose rings can be handled within the mathematical framework developed in DAS for molecules with tree topology.

OPTIMIZATION

Conformational energy and various objective functions of a macromolecule have a general form given by

$$E(\theta_1, \theta_2, \dots, \theta_n) = \sum_a \Psi_a(\theta_a) + \sum_\alpha \sum_\beta \Phi_{\alpha\beta}(r_{\alpha\beta}) \quad (4)$$

Terms of the first type are explicit functions of dihedral angles. Their first and second derivatives with respect to dihedral angles can be easily calculated. The torsional potential about rotatable bonds in a conformational energy function belongs to this type. Torsional angle constraints derived from NMR J -coupling data in an objective function for

TABLE I.
Values of the Parameters a_j , b_j , and c_j in Eqs. (2) and (3).

	$j = 0$	$j = 4$	$j = 3$	$j = 2$	$j = 1$
Ribose					
a_j	-3.302×10^{-1}	-2.596×10^{-1}	-2.159×10^{-1}	-2.203×10^{-1}	-2.593×10^{-1}
b_j	2.069×10^{-2}	3.128×10^{-2}	4.162×10^{-2}	4.036×10^{-2}	2.793×10^{-2}
c_j	0.000	5.515×10^{-2}	1.685×10^{-2}	5.140×10^{-2}	-7.830×10^{-2}
Deoxyribose					
a_j	-3.325×10^{-1}	-2.551×10^{-1}	-2.323×10^{-1}	-2.171×10^{-1}	-2.668×10^{-1}
b_j	2.364×10^{-2}	2.891×10^{-2}	3.531×10^{-2}	3.357×10^{-2}	2.676×10^{-2}
c_j	0.000	3.225×10^{-2}	1.130×10^{-2}	-3.840×10^{-2}	5.035×10^{-2}

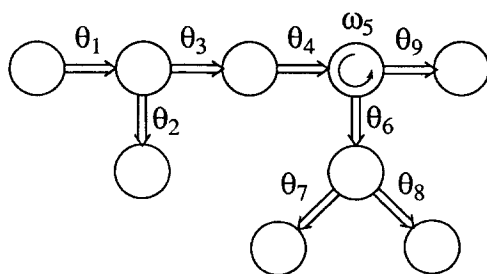


FIGURE 1. Schematic representation of part of a polynucleotide chain. Rotatable bonds are indicated by arrows. Circles denote rigid units composed of one or more atoms. The circle with an arrow in it denotes a variable unit, i.e., a furanose ring, where ω is the pseudorotation variable.

3D structure determination also belong to this type. Terms of the second type are functions of interatomic distances. Coulomb-type electrostatic and Lennard-Jones-type nonbonded energy functions in a conformational energy function belong to this type. Interatomic distance constraints in an objective function for structure determination belong to this type. These terms are implicit functions of dihedral angles, because interatomic distances are given as functions of dihedral angles. It is not trivial to calculate efficiently the first and second

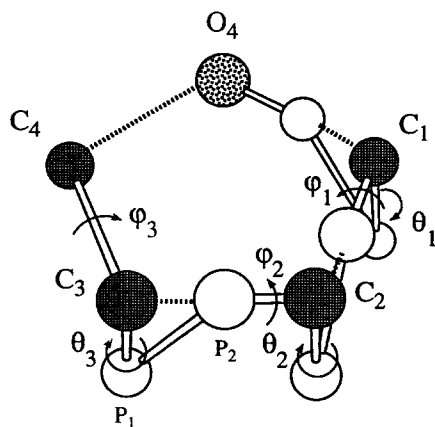


FIGURE 2. Introduction of pseudoatoms into a furanose ring. Open circles, meshed circles, and a spotted circle represent pseudoatoms, carbon atoms, and an oxygen atom, respectively. The meaning of the pseudoatoms marked P_1 and P_2 is described in the text. Dashed lines denote bonds which are conceptually cut. Bonds marked with φ indicate those with rotatable dihedral angles; others marked with θ indicate bonds with virtual rotation used to describe variation of bond angles. All of them are variables dependent on the pseudorotation variable ω .

derivatives of these terms with respect to dihedral angles for macromolecules.

For molecules with a tree topology a rapid algorithm for computation of the derivatives has been developed.^{7,28,29} We now consider an extension of the algorithm so that nucleic acids with flexible furanose rings can be treated. For nucleic acids, since we have two types of independent variables, the following six types of operators should be considered:

$$\frac{\partial}{\partial \theta_a^{\text{ind}}}, \quad \frac{\partial^2}{\partial \theta_a^{\text{ind}} \partial \theta_b^{\text{ind}}}, \quad \frac{\partial}{\partial \omega_a}, \quad \frac{\partial^2}{\partial \theta_a^{\text{ind}} \partial \omega_b},$$

$$\frac{\partial^2}{\partial \omega_a \partial \omega_b}, \quad \frac{\partial^2}{\partial \omega_a^2} \quad (5)$$

where θ^{ind} is a dihedral angle which is an independent variable and ω is a pseudorotation variable (Figure 1). The first two types of operators are those considered already in the existing algorithm, while the remaining four types are new and are involved in the pseudorotational motion of furanose rings. Now, according to eq. (1), they can be written down, respectively, as follows:

$$\frac{\partial}{\partial \omega_a} = \sum_{i=1}^6 \left(\frac{\partial \theta_{ai}^{\text{dep}}}{\partial \omega_a} \right) \frac{\partial}{\partial \theta_{ai}^{\text{dep}}} \quad (6)$$

$$\frac{\partial^2}{\partial \theta_a^{\text{ind}} \partial \omega_b} = \sum_{i=1}^6 \left(\frac{\partial \theta_{bi}^{\text{dep}}}{\partial \omega_b} \right) \frac{\partial^2}{\partial \theta_a^{\text{ind}} \partial \theta_{bi}^{\text{dep}}} \quad (7)$$

$$\frac{\partial^2}{\partial \omega_a \partial \omega_b} = \sum_{i=1}^6 \sum_{j=1}^6 \left(\frac{\partial \theta_{ai}^{\text{dep}}}{\partial \omega_a} \right) \left(\frac{\partial \theta_{bj}^{\text{dep}}}{\partial \omega_b} \right) \frac{\partial^2}{\partial \theta_{ai}^{\text{dep}} \partial \theta_{bj}^{\text{dep}}} \quad (8)$$

$$\frac{\partial^2}{\partial \omega_a^2} = \sum_{i=1}^6 \sum_{j=1}^6 \left(\frac{\partial \theta_{ai}^{\text{dep}}}{\partial \omega_a} \right) \left(\frac{\partial \theta_{aj}^{\text{dep}}}{\partial \omega_a} \right) \frac{\partial^2}{\partial \theta_{ai}^{\text{dep}} \partial \theta_{aj}^{\text{dep}}} + \sum_{i=1}^6 \left(\frac{\partial^2 \theta_{ai}^{\text{dep}}}{\partial \omega_a^2} \right) \frac{\partial}{\partial \theta_{ai}^{\text{dep}}} \quad (9)$$

where θ^{dep} is a variable which is dependent on a pseudorotation variable. These operators are composed of two types of factors: differentiations of the dependent variables with respect to the pseudorotation variable, and first and second derivatives with respect to the dependent variables. Factors of the former type can be easily calculated from eq. (1). Since factors of the latter type have the same forms as the first and second derivatives with respect to independent dihedral angles, which are the first two types of operators in eq. (5), they can be calculated rapidly by treating the depen-

dent variables as independent variables in the existing algorithm.

Consequently, computation of the first and second derivatives should be carried out by the following procedure:

1. Values of dependent variables in furanose rings are calculated by functions of pseudorotation variables as in eq. (1).
2. The first and second derivatives, $\partial E/\partial\theta_a$ and $\partial^2 E/\partial\theta_a \partial\theta_b$, are computed for all independent and dependent rotatable dihedral angles by the rapid computational algorithm.
3. Differentiations of the dependent variables with respect to the pseudorotation variable $\partial\theta^{\text{dep}}/\partial\omega$ are calculated according to eq. (1).
4. The first and second derivatives with respect to the pseudorotation variable ω are calculated by eqs. (6), (7), (8), and (9).
5. All first and second derivatives for independent variables in eq. (5) are calculated.

Thus, the first and second derivatives with respect to dihedral angles, all independent variables, and pseudorotation variables, can be calculated efficiently.

VARIABLE TARGET FUNCTION

In the present study, we apply the rapid computational algorithm to a distance geometry calculation based on the variable target function method. A target function T_k , which measures how well a present conformation satisfies experimental distance constraints, is defined as follows:

$$\begin{aligned}
 T_k = & \frac{w_u}{4} \sum_{(\alpha, \beta)} \rho_k(\alpha, \beta) \Theta(d_{\alpha\beta} - u_{\alpha\beta}) \\
 & \times (d_{\alpha\beta}^2 - u_{\alpha\beta}^2)^2 / u_{\alpha\beta}^2 \\
 & + \frac{w_l}{4} \sum_{(\alpha, \beta)} \rho_k(\alpha, \beta) \Theta(l_{\alpha\beta} - d_{\alpha\beta}) \\
 & \times (l_{\alpha\beta}^2 - d_{\alpha\beta}^2)^2 / l_{\alpha\beta}^2 \\
 & + \frac{w_s}{4} \sum_{(\alpha, \beta)} \rho_k(\alpha, \beta) \Theta(s_{\alpha} + s_{\beta} - d_{\alpha\beta}) \\
 & \times \{(s_{\alpha} + s_{\beta})^2 - d_{\alpha\beta}^2\}^2 \\
 & + \frac{w_t}{4} \sum_a \left\{ 1 - \left(\frac{\theta_a - \theta_a^m}{\theta_a^w} \right)^2 \right\}^2 \quad (10)
 \end{aligned}$$

Here $u_{\alpha\beta}$ and $l_{\alpha\beta}$ are given upper and lower bounds of distance $d_{\alpha\beta}$ between atoms α and β , respectively; s_{α} and s_{β} are repulsive core radii of the two atoms; bounds of dihedral angles θ_a are given by $\theta_a^m + \theta_a^w$ and $\theta_a^m - \theta_a^w$, where θ_a^m is a target value and θ_a^w is a width of bounds; w_u , w_l , w_s , and w_t are weight factors; Θ is the step function defined as $\Theta(x) = 1$ for $x \geq 0$ and $\Theta(x) = 0$ for $x < 0$; k is a distance between atoms along the chain, which is defined as the number of rotatable dihedral angles between them ($0 \leq k \leq k_{\text{max}}$, k_{max} is maximum distance along the chain); $\rho_k(\alpha, \beta) = 1$, if distance between atoms α and β along the chain is equal to or smaller than the value of k , and $\rho_k(\alpha, \beta) = 0$ otherwise. The summations in the first three terms are taken over all pairs of atoms, and the one in the last term is taken over all dihedral angles.

The most satisfactory conformation corresponds to the global minimum of the target function $T_{k_{\text{max}}}$. However, it is usually difficult to obtain the global minimum by simple minimization of $T_{k_{\text{max}}}$. In order to avoid becoming trapped in a local minimum, the minimization of the target function is carried out with gradual increase of the value of k .⁸

Results and Discussion

In the present study, we apply the variable target function method to an RNA hairpin loop whose sequence is 5'GGAC(UUCG)GUCC. This is a very common RNA hairpin. The loop sequence UUCG occurs exceptionally often in ribosomal and other RNAs. Moreover, molecules with this hairpin loop display unusually high thermodynamic stability.³⁰ Three-dimensional conformations of this hairpin loop in solution have been determined by Tinoco and co-workers from 415 NMR-obtained constraints^{31,32} and by the distance geometry program DSPACE⁴ that works in CCS. Then, refinement was performed by repeating cycles of simulated annealing with the AMBER potential.³ Ten conformations were thus determined which best satisfied the constraints.³² These distance constraints and the 10 conformations were kindly given to us by Professor I. Tinoco, Jr.; the 10 conformations are reproduced in Figure 3b.

We carry out our optimization in DAS starting from 80 randomly generated starting conformations for the same set of distance constraints. Ten conformations with the smallest residual values of the target function are determined. Then they are

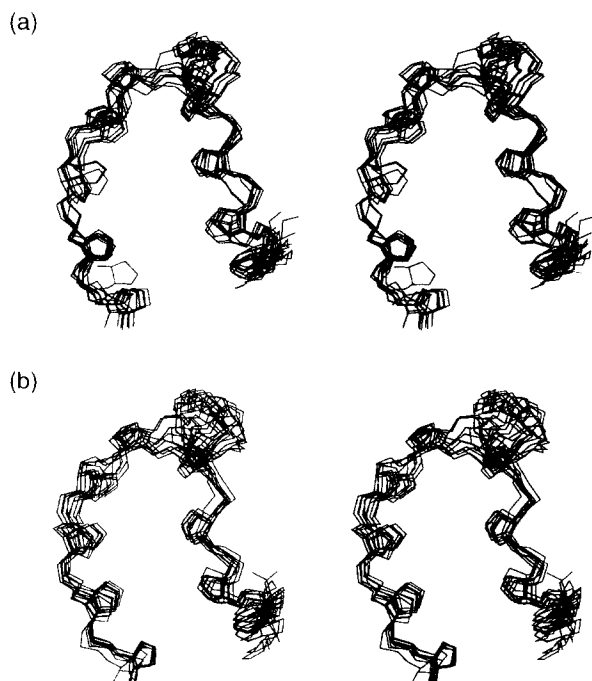


FIGURE 3. Backbone heavy-atom mutually superimposed stereo view of the 20 conformations. (a) Ten conformations that are determined in DAS. (b) Ten conformations that were determined in CCS.

also subjected to potential energy minimization with the AMBER potential together with the distance constraints. Obtained conformations are shown in Figure 3a. Root-mean-square distance values⁸ of all pairs of the determined conformations are given in Table II. Two points should be noted. One is that the values for the DAS conformations are larger than those for the CCS conformations. This indicates that the DAS conformations are more widely spread. This fact can be observed intuitively by comparing Figure 3a and b. Another is that the values between the DAS and the CCS conformations are much larger than both of those for the DAS conformations and for the

TABLE II.
Averages of Root-Mean-Square Distances (angstroms) between Two Sets of Conformations Obtained in DAS and in CCS, Respectively.

	All Heavy Atom		Backbone Heavy Atom	
	DAS	CCS	DAS	CCS
DAS	1.530	1.965	1.475	1.882
CCS		1.452		1.410

CCS conformations. This indicates that an average of the DAS conformations is somewhat displaced from that of the CCS conformations. To evaluate the quality of each of the conformations determined by the two methods, we calculate residual violations defined by

$$V = \sum_{(\alpha, \beta)} \left\{ \Theta(d_{\alpha\beta} - u_{\alpha\beta})(d_{\alpha\beta} - u_{\alpha\beta})^2 + \Theta(l_{\alpha\beta} - d_{\alpha\beta})(l_{\alpha\beta} - d_{\alpha\beta})^2 \right\} \quad (11)$$

Results are shown in Table III. The number of major violations ($> 0.5 \text{ \AA}$) are also shown in Table III. In DAS, all conformations have similar small values, whereas in CCS almost all conformations but two have relatively large values. When a conformation satisfies the set of distance constraints perfectly, the value of residual violation should vanish. In Table III, we see that all conformations obtained in DAS have about 5 \AA^2 for the residual violation. In order to find the reason for this rather large value of the residual violation, we have done a further analysis to identify major contributions to the residual violations. Unexpectedly, we found that in all of the 10 conformations there were about 10 major violations ($> 0.5 \text{ \AA}$). All of these major violations occur in some of 11 constraints between the P atom in phosphate groups and the H5' atom in the neighboring bases. These two atoms are in the so-called 1-4 connectivity, i.e., connected via O and C atoms as $\text{P}-\text{O}5'-\text{C}5'-\text{H}5'$. Their interatomic distance varies between 3.54 and 2.62 \AA according to rota-

TABLE III.
Residual Violations (Resid; in angstroms squared) and the Number of Major Violations (No.; $> 0.5 \text{ \AA}$) in Two Sets of 10 Conformations Obtained in DAS and in CCS, Respectively.

Serial Number of Structures	DAS		CCS	
	Resid.	No.	Resid.	No.
1	4.898	(9)	0.004	(0)
2	5.573	(10)	0.013	(0)
3	5.793	(10)	10.513	(17)
4	5.811	(10)	11.164	(18)
5	5.815	(9)	11.936	(19)
6	5.845	(9)	12.018	(18)
7	5.862	(9)	12.494	(20)
8	6.072	(11)	12.915	(18)
9	6.097	(10)	13.128	(18)
10	6.137	(9)	14.800	(21)

tion about O5'—C5' bond. However, out of the 11 upper bound interatomic distance constraints assigned by the original authors between the P atom and the H5' atom, seven took a value of either 2.05 or 2.10 Å, which leads inevitably to violation larger than 0.5 Å, and three either 2.15 or 2.20 Å. More than 70% of the residual violation in the 10 DAS conformations were found to come from these 10 constraints. The CCS calculation successfully generated two conformations which have nearly zero residual violations. These two conformations were found to have unusual O5'—C5'—H5' bond angles. The other eight conformations with more standard bond angles have large residual violations.

Thus, the results shown in Tables II and III indicate that the conformations are sampled from subspace, which better satisfies the set of distance constraints more evenly in DAS than in CCS. Accordingly, the CCS conformations are narrowly distributed in the conformational space slightly displaced from the good subspace.

Values of pseudorotation variable ω in the conformations determined by the two methods are shown in Figure 4. We see that the values obtained by the two methods are well correlated. This supports the reasonableness of the treatments of pseudorotation in the two methods. It should be noted that only a few distance constraints involve atom pairs existing in the same furanose rings. Therefore, the observed puckering states of furanose rings are not direct consequences of the imposed distance constraints, but are consequences of overall conformations of the molecule, which are con-

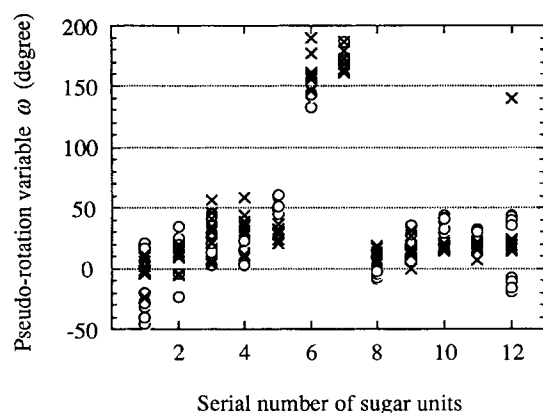


FIGURE 4. Values of the pseudorotation variable ω for all furanose rings: Crosses indicate the values in conformations determined by Varani et al. in CCS; circles indicate those in conformations determined in this paper in DAS.

sequences of the imposed constraints. This close correlation between the overall conformations and puckering in furanose rings indicates that good treatment of furanose ring puckerings is essential for conformational study of polynucleotide chains.

Accordingly, our algorithm has enough ability to determine three-dimensional conformations of nucleic acids from a set of distance constraints without losing computational efficiency. The algorithm is coded into a computer program by FORTRAN77. The program runs on a supercomputer and on a UNIX workstation. In the future we will extend the algorithm so as to handle double-stranded nucleic acid molecules.

Acknowledgments

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